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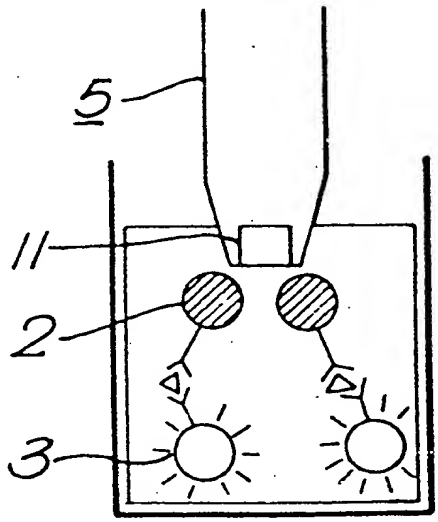
(54) Title: METHOD AND DEVICE FOR CARRYING OUT IMMUNOLOGICAL ASSAYS



(57) Abstract

Method and equipment for carrying out immunoassays, wherein to a solution containing the antibody to be determined, magnetic particles (2) coated with the corresponding antigen as well as tracer particles (3) coated with the corresponding antigen are added, after the immunological reaction the magnetic particles and the tracer particles adhering to them by the intermediate of the antibody are separated from the reaction solution, and the radiation emitted by the separated particles is measured. The magnetic particles are separated from the reaction solution by pushing a magnetic piece (5) into the solution and by pulling it out of the solution after the magnetic particles have adhered to it, whereupon the radiation emitted by the separated particles is measured.

Method and device for carrying out immunological assays

The present invention is concerned with a fluorometric or phosphorimetric immunoassay method in which small polymer particles are used as the solid phase. The method in accordance with the invention can be used, besides for immunoassays in general, also for blood group determinations.

In prior art, methods are known which are based on immobilization of an antibody or antigen on an antigen or antibody in advance placed on a solid face as well as on the use of an antibody or antigen labelled with a tracer. Such methods are, e.g., RIA (Radioimmunoassay) and SP-FIA (Solid Phase Fluoroimmunoassay). In all of these methods, the solid face on which the immunological reaction has taken place and the reaction solution must be separated from each other before the signal of the tracer is measured in order that the excess tracer present in the reaction solution should not cover the signal of the tracer present in the antibody or antigen immobilized on the solid phase. The signal concerned may be, e.g., radioactivity (RIA), fluorescence signal (FIA) or even enzyme activity (EIA).

The separation of the solid phase from the reaction solution always includes washing of the solid phase, which at present, as a rule, requires manual operations. If small polymer particles are used, like in the method of the present invention, these operations include centrifuging or magnetic deposition.

The object of the present invention is to provide a simple manual method for the determination of antibodies or antigens, which said method is also suitable for use with such antibodies or antigens as are placed on the surface of cells or other particles of organic origin.

In the assay method in accordance with the invention, tracer that emits radiation, which said tracer

may be in soluble form or preferably on polymer particles, is, together with particles that contain a magnetic material, immobilized on the antibody (or antigen) to be determined by means of an immunological bond. After the immobilization, the magnetic particles and everything that has been immobilized on them by means of the immunological bonds are pulled onto the face of a magnet to be submerged into the reaction solution. The magnet belonging to the apparatus in accordance with the invention is preferably placed at the end of a rod, and the magnet is preferably provided with a protective cover, onto which the particles become positioned. The rod and the particles ahdering to it are pulled out of the reaction solution, and thereinafter the rod can be submerged into any washing and fixing solutions that may be needed. Finally, the rod with the particles adhering to it are, if desired, provided with a second protective cover and placed in a reading apparatus in accordance with the invention, wherein the radiation from the particle mass is measured in a way in itself known. The protective covers or one of them may be, in advance, provided with a substance affecting the intensity of the radiation signal, such as, e.g., a substrate of the enzyme used as the tracer, which substrate is made fluorescent by the enzyme.

A preferred exemplifying embodiment of the invention with its apparatuses is illustrated by means of the accompanying figures.

Figure 1 illustrates a reaction solution, in which, besides the sample to be studied, there are also magnetic and fluorescent particles.

Figure 2 illustrates pulling of the particle mass onto the end of the magnetic rod.

Figure 3 shows the reading apparatus.

Figure 4 shows the rod with the magnet and with the protective covers attached to it.

Figure 5 shows a protective cover to be placed

on the magnet.

A sample which contains antibody 1 and which has been diluted appropriately is placed in a reaction vessel 6 (Fig. 1). Magnetic particles 2 and fluorescent particles 3 are also administered into the vessel, both of which have been coated with an antigen 4 corresponding to the antibody 1. Ordinary incubation is carried out. The antibody 1 to be determined adheres both to the magnetic particles 2 and to the fluorescent particles 3, also causing adherence of these particles to each other. Without the intermediate of the antibody, the magnetic and the fluorescent particles cannot adhere to each other. Upon completion of the reaction, a magnetic rod 5 (Fig. 2) is submerged into the reaction solution. The magnetic particles 2 and the fluorescent particles 3 possibly adhering to them are collected by the effect of the magnet 11 onto the end of the rod 5. Hereupon the rod 5 is lifted off the reaction solution and submerged in a washing solution.

After washing, the rod 5 is placed into a measurement opening 8 provided in the reading apparatus 7 (Fig. 3), in which said opening the fluorescence of the particle mass is measured by using an excitation light of suitable colour and by detecting the fluorescent radiation emitted from the particle mass.

Figure 4 shows the magnetic rod to be used in particular in the method. The rod comprises a tubular outer sleeve 9, composed of two parts connected to each other, and an inner rod 10 gliding in the sleeve. At the bottom end of the inner rod 10, there is a permanent magnet 11.

The bottom end of the rod is conical, and onto the bottom end cup-shaped protective covers 12 and 13, placed one inside the other, have been pressed by means of a friction joint. The inner cover 12 is placed into its position before the rod is submerged into the reaction solution, and the outer cover 13 is pushed onto

the inner cover after the washing stage. Thus, the particles adhering to the rod from the reaction solution remain between the protective covers 12 and 13. The protective covers are preferably disposable, and by their use it is possible to prevent contamination and wetting of the rod proper, on one hand, and of the measurement apparatus, on the other hand. The protective covers joined together can also be detached from the rod before measurement.

The top end of the inner rod 10 extends to outside the outer sleeve 9, thus forming a press knob 14 by whose depression the covers 12 and 13 can be detached. Around the inner rod 10, a spiral spring 16 is fitted between the annular flange 15 and the bottom tip of the outer sleeve, which said spring pushes the inner rod to its upper position. The outer sleeve 9 is further provided with a limiter flange 17, and the inner rod with a shoulder 18, which prevent removing of the inner rod out of the sleeve.

Figure 5 is a more detailed view of the protective cover used on the rod. The cover is a cup made of a suitable material not interfering with the measurement, the bottom of the said cup being provided with feet 19. Into the cover, it is possible to place a substance affecting the fluorescence signal. This substance may be, e.g., a substrate of the enzyme used as the tracer, which substrate is made fluorescent by the enzyme. If desired, a fluorescent substance may also be placed into the cover, for which substance the radiation emitted from the sample acts as excitation radiation. In this way it is possible to transfer the signal to a longer, more readily detectable wavelength.

In stead of a rod, it is also possible to use an object of some other form which is provided with a magnet.

The magnet may also be an electric magnet, in which case the rod must, of course, be provided with the

necessary connection for the supply of electricity. Such an embodiment may be concerned if it is desirable to eliminate the magnetic field in between.

The reading apparatus may be provided with automatic means which start the measurement immediately after the rod has been inserted into the measurement opening. The measurement equipment itself comprises a source of light, from which the excitation radiation is passed to the sample, a detector, into which the emission radiation is passed, as well as the necessary optics and equipment for the processing and display of the measurement result.

If desired, the reaction vessel can be shaped such that the rod can be pushed into it only up to a certain depth, whereby only the protective cover placed at the end of the rod becomes wet.

The substance to be determined may, of course, be an antibody equally well as an antigen, and the radiation of the tracer may be, e.g., phosphorescent or radioactive radiation.

WHAT IS CLAIMED IS:

1. Method for carrying out immunoassays, in which said method to a solution containing the antibody (1) to be determined, magnetic particles (2) coated with the corresponding antigen (4) as well as tracer particles (3) emitting radiation and coated with the corresponding antigen are added, after the immunological reaction the magnetic particles and the tracer particles adhering to them by the intermediate of the antibody are separated from the reaction solution, and the radiation emitted by the separated particles is measured, c h a r a c t e r i z e d in that the magnetic particles are separated from the reaction solution by pushing a magnetic piece (5) into the solution and pulling it out of the solution after the magnetic particles have adhered to it, whereupon the radiation emitted by the separated particles is measured.

2. Method as claimed in claim 1, c h a r a c t e r i z e d in that before the magnetic piece is inserted into the reaction solution, an inner protective cover (12) is attached to the piece, which said cover prevents contamination of the magnetic piece.

3. Method as claimed in claim 1, c h a r a c t e r i z e d in that the radiation emitted by the separated particles is measured when the particles still adhere to the magnetic piece and that, before measurement, an outer protective cover (13) is attached onto the magnetic piece, which said cover (13) prevents contamination of the measurement apparatus used.

4. Method as claimed in claim 2, c h a r a c t e r i z e d in that, before the radiation is measured, an outer protective cover (13) is attached onto the inner protective cover (12).

5. Equipment for carrying out the method as claimed in claim 1, w h i c h comprises a reaction vessel (6), a magnetic piece (5) to be inserted into

the reaction vessel, as well as a measurement apparatus (7) for the measurement of the fluorescence of the particles adhering to the magnetic piece.

6. Equipment as claimed in claim 5, c h a r - a c t e r i z e d in that the magnetic piece is a rod (5), whose bottom tip is provided with a magnet (11).

7. Equipment as claimed in claim 6, c h a r - a c t e r i z e d in that the magnet is a permanent magnet (11).

8. Equipment as claimed in claim 6, c h a r - a c t e r i z e d in that the rod (5) is provided with a tubular outer sleeve (9) and with an inner rod (10) placed inside the said sleeve, the bottom tip of the said inner rod being provided with a magnet (11), and that the inner rod can move at least a certain distance to underneath the bottom tip of the outer sleeve.

9. Equipment as claimed in claim 8, c h a r - a c t e r i z e d in that the rod (5) is provided with a spring (16), which presses the upper rod (10) to its upper position.

10. Equipment as claimed in claim 6, c h a r - a c t e r i z e d in that the bottom tip of the rod (5) is conical.

INTERNATIONAL SEARCH REPORT

International Application No PCT/FI86/00041

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC 4

G 01 N 33/553

II. FIELDS SEARCHED

Minimum Documentation Searched †	
Classification System	Classification Symbols
IPC	G 01 N 33/53, /543, /553
US Cl	436:500, 501, 526, 527, 537

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ‡

SE, NO, DK, FI classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT *

Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
Y	US, A, 4 115 535 (GIAEVERI) 19 September 1978	1, 5
Y	US, A, 4 272 510 (SMITH K.O. ET AL) 9 June 1981 & US, 4292920	1, 5-7
P	EP, A2, 140 787 (INSTITUT PASTEUR) 8 May 1985	1, 5-7
A	EP, A2, 136 126 (CORNING GLASS WORKS) 3 April 1985	1, 5
A	Methods in Ensymology, Immunochemical techniques, Part B, Vol 73 pages 471-82 (Ed. Langone J.J. et al)	1, 5
A	US, A, 3 981 776 (SAXHOLM R) 21 September 1976	5

* Special categories of cited documents: ¹⁰

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
1986-07-29	31 JULY 1986
International Searching Authority	**Signature of Authorized Officer**
Swedish Patent Office	Carl Olof Gustafsson

Form PCT/ISA/210 (second sheet) (January 1985)

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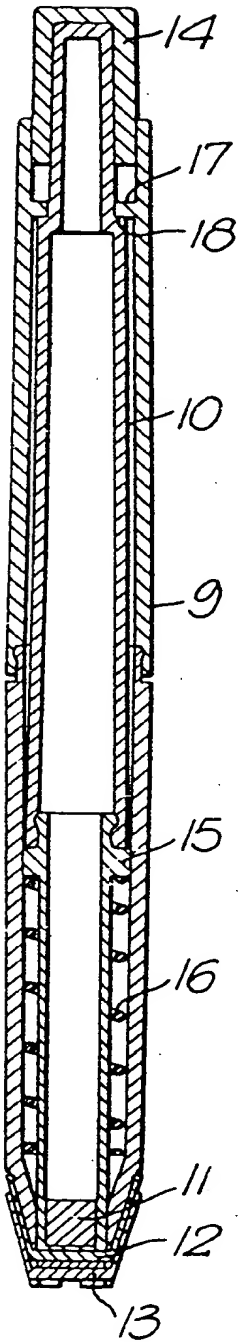
Fig. 4.



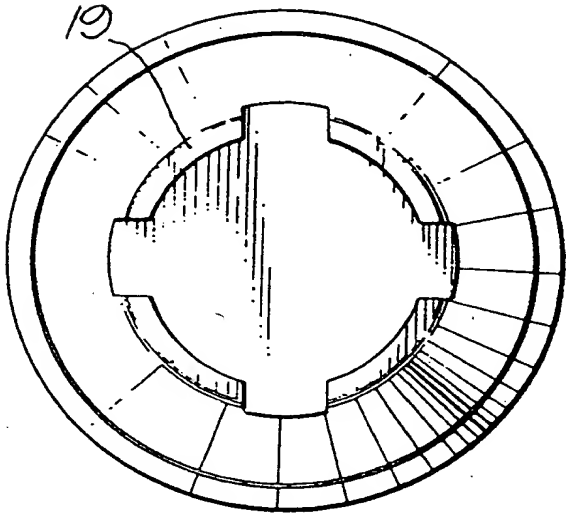


Fig. 5.

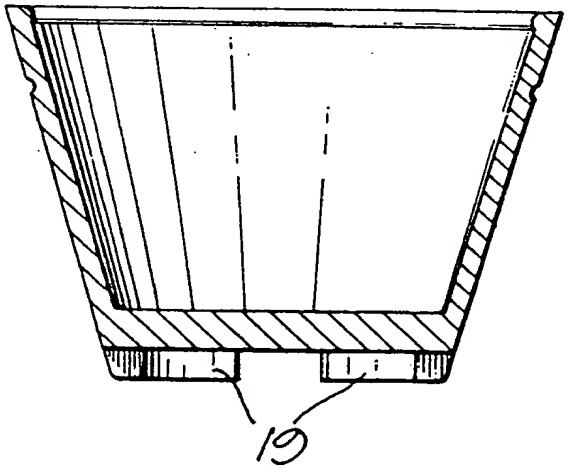


Fig.1.

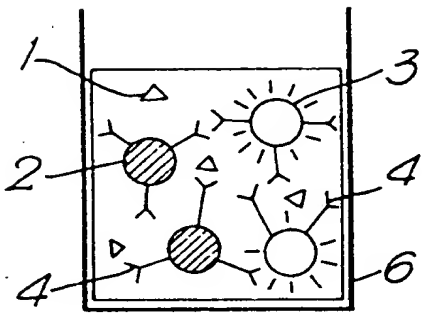


Fig.2.

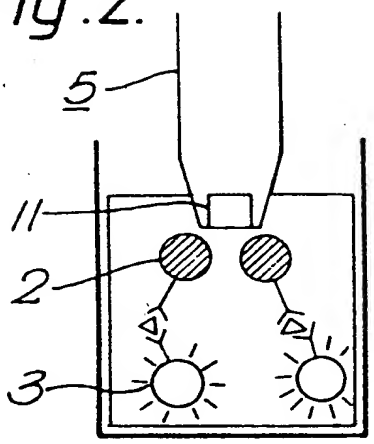


Fig.3.